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Fertilization in Fritillaria pudica

Karl Sax

(WITH PLATES 27–29 AND THREE TEXT-FIGURES)

Fertilization in the Angiosperms and especially in the Liliaceae has been the subject of research of many botanists. Yet it is surprising how incomplete and fragmentary is our knowledge of this important and interesting process.

Strasburger (13) in 1884 described fertilization in *Monotropa Hypopitys*. He observed the streaming of the cytoplasm in the embryo sac in the living condition and suggested that the male nucleus was carried to the egg by cytoplasmic streaming.

Guignard (4) in 1891 described fertilization in *Lilium Martagon* and showed a large number of fertilization stages.

In 1897 Mottier (8) first described the vermiform shape of the male nuclei in *Lilium Martagon*.

In 1899 Guignard (5) and Nawaschin (10) published independently their discovery of double fertilization in *Lilium* and *Fritillaria*.

Nawaschin (10, 11, 12) has described fertilization in *Lilium Martagon*, *Fritillaria tenella*, *Juglans nigra* and *Helianthus annuus*. In *Lilium Martagon* and *Fritillaria tenella* he describes the vermiform male nuclei and concludes that they are motile. He maintains that the male nuclei are discharged into the space between the egg apparatus and the "Endospermanlage," whence they make their way to the egg and polar nucleus. He believes that the male nuclei must be motile in order to pass from one

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medium to another and to penetrate the membranes of the egg cell and the "Endospermanlage." In one case he finds the male nuclei linked together and attacks Strasburger's (13) theory of cytoplasmic streaming on the ground that streaming could not carry these nuclei in different directions. He also notes that the bodies, the "X-Körper," which accompany the male nuclei in the pollen tube, are not carried with the male nuclei toward the egg or upper polar nucleus. He suggests that in *Helianthus annuus* one synergid penetrates the embryo sac and pours its contents into the micropyle canal and thereby aids in the entrance of the pollen tube. The cases of fertilization found were apparently few.

In 1900 Guignard (6) published his paper on fertilization in the tulip. He describes the male nuclei in the pollen tube as being of curved shape and of almost homogeneous structure. On leaving the tube they are accompanied by a dense granular substance. The pollen tube closes after the male nuclei escape.

Blackman and Welsford (1) in 1913, working on *Lilium Martagon* and *L. auratum*, describe and picture vermiform male nuclei both in the contents of the pollen tube and attached to the egg and polar nuclei. They also mention and show the two dark bodies in the end of the pollen tube. The male nuclei pictured are long vermiform and often pointed at one end, and their contents are arranged in a rather regular network. The authors maintain that the first male nucleus is smaller and suggest that, since the smaller male nucleus is found in contact with the egg, the first male nucleus goes to the egg. They also believe that the male nuclei are motile.

Welsford (14) believes that the "X-Körper" of Nawaschin are the disintegrating cytoplasm which surrounded the male nuclei. Mention is also made that the pollen tube itself enters the embryo sac before the discharge of the contents.

Other writers have published more or less complete descriptions of fertilization in various Angiosperms. But nowhere is there so complete a description of fertilization in the Angiosperms as has been published by Miss Ferguson (2, 3) and others for the Gymnosperms. Miss Ferguson describes a complete series of egg and sperm fusions in *Pinus strobus* and finds the interesting fact that the sexual nuclei do not completely fuse but form independent spindles in close proximity to each other.

The material for the present study was collected near Pullman, Washington, in April and May, 1914 and 1915. *Fritillaria pudica* Spreng. is exceptionally good for the study of the process of fertilization because it seeds so freely. In many ovaries practically every sac contained a stage in fertilization. In several ovaries stages were found from synapsis of the megaspore mother cell to stages in fertilization. In the material cut and stained I have found not less than two thousand cases of double fertilization at various stages.

Strong chrom-acetic acid and Flemming's stronger solution were used as fixatives. Most of the sections were cut 10 μ thick, but a few were cut 15 μ and 20 μ. They were stained with modifications of Flemming's triple stain and with safranin and Lichtgrün. Good results were obtained with all stains used.

Although the development of the ovule has been traced from the stage containing the megaspore mother cell up to the time when the young embryo and endosperm are formed, this paper will deal largely with fertilization. Other processes of interest will be dealt with in a later paper.

The mature embryo sac of *Fritillaria pudica* contains the egg and two synergids at the micropylar end, and one normal antipodal cell and one which is disorganized at the chalazal end. Between these groups of cells is the "Endospermanlage" of Nawaschin, which contains the two polar nuclei, the upper one near the egg apparatus, the lower one near the antipodal end. There are thus usually but seven nuclei in the mature embryo sac. The one nearest the chalazal end is at least partly disorganized. The lowest of the four nuclei resulting from the second division usually fails to divide. The three cells of the egg apparatus are somewhat pear-shaped and arranged symmetrically in the upper end of the sac. They are usually very much alike in size and appearance and it is very doubtful if the egg can be distinguished from the other two cells until the time of fertilization. They are bounded by very delicate membranes, which shows them to be distinct cells. Their cytoplasmic contents are less dense and stain lighter than those of the "Endospermanlage" and antipodal cells. At each end of the "Endospermanlage" is a distinct membrane. At the micropylar end the " Endospermanlage " may project up between or around the cells of the egg apparatus. There is a great deal of variation in this respect, the "Endospermanlage" extending up around the cells of the egg apparatus at different points to varying distances. The upper polar nucleus is smaller and less regular in outline than the lower one. It is always located near the periphery of the sac and surrounded by less cytoplasm. The central portion of the " Endospermanlage " consists of a large vacuole and a thin peripheral layer of cytoplasm. The thickness of this layer is considerably less than the upper polar nucleus (TEXT FIG. 1).

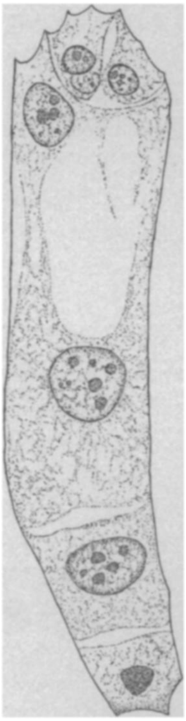

FIG. 1. Mature embryo sac showing: the egg apparatus, one cell of which is only partly visible; the "Endospermanlage" containing the two polar nuclei, the upper one near the egg apparatus; one normal antipodal cell and one which is disorganizing.

Only one case was observed where the tube had reached the embryo sac but had not penetrated it. The tip of the tube was enlarged and rounded and had turned along the nucellus towards one of the nuclei of egg apparatus, probably that of a synergid. In practically all cases one of the synergids is destroyed by the entering pollen tube; but just what the relationship between the synergid and the pollen tube is could not be determined. In one case the cell as well as the nucleus in it, through which the pollen tube is about to enter, has a pointed end towards the end of the pollen tube. But two, or even all of the cells and nuclei of the egg apparatus, may be more or less pointed towards the micropyle. When the pollen tube is in contact with the embryo sac, the cells of the egg apparatus are all intact and show no signs of having emptied their contents into the micropyle, or even of having penetrated the wall of the embryo sac.

In most cases the pollen tube enters the embryo sac somewhat from the side after turning from the micropyle, but sometimes it penetrates the nucellus and embryo sac directly from the micropyle.

The pollen tube just after entering the embryo sac is rounded and swollen at the tip. Only one case was found at this stage but it was well fixed, very clearly stained and apparently normal. The pollen tube and embryo sac are cut longitudinally. The pollen tube contains the two male nuclei. They stain darkly and are much elongated. Two other nuclei are also seen in the sections of the tube. The male nucleus (*a*) nearest the tip shows in FIGS. 3A and 3B. It is long and much coiled and consequently cut across at several places. The second male nucleus (*b*) is seen mostly in FIG. 3C, but the ends of the nucleus are seen in FIG. 3B. This male nucleus is not so long and coiled as the other. The second male nucleus and perhaps the first one are somewhat pointed at the end. The contents at this stage are in an irregular network which takes a deep red stain with saffranin. The chromatin is not entirely peripheral as is shown where the male nuclei are seen in section. To the right in FIG. 3C is a nucleus (*d*) of practically the same size, shape and appearance as the synergid nuclei. The nucleus of the synergid into which the pollen tube has passed, is not found elsewhere in the embryo sac. To the left in FIG. 3C is a second vegetative nucleus (*c*) which is flattened and irregular in shape and situated between the two male nuclei. Portions of these vegetative nuclei are seen in FIG. 3B. FIG. 3A contains some dark-staining granules. The pollen tube did not stain nearly as darkly as others in the same ovary where the male nuclei had already escaped. It is questionable whether both of the vegetative nuclei, (*c*) and (*d*), are tube nuclei, or whether the one to the right (*d*) is the synergid nucleus which has been surrounded by the pollen tube.

The examples of stages in fertilization described so far are comparatively few. But after the male nuclei leave the pollen tube, numerous examples of the stages are found. Not less than eighty male nuclei were found free in the cytoplasm of the cells of the embryo sac, and typical cases of these are figured.

On leaving the pollen tube the male nuclei apparently retain their curved or coiled shape for some time and in many instances it is probable that they never lose their coiled or curved shape during migration. When the male nucleus escapes near the nucleus with which it is about to fuse, which is always true in the case of the egg, it is probable that it seldom loses its coiled shape, although cases were observed where it was practically straightened out. The male nucleus going to the upper polar nucleus is more frequently straightened out than is the case with the one going to the egg nucleus, probably due to the greater distance it has to travel.

A clear example of the male nucleus going to the upper polar nucleus is shown in FIG. 2. The upper polar nucleus is at the right of the "Endospermanlage." The free male nucleus is distinctly pointed at one end and is curved in a manner that gives it the appearance of motility. The end toward the upper polar nucleus is larger, rounded and tapers slightly toward the middle, where it forms a short rounded arch, and then curves to one side and tapers to a point.

FIG. 1 shows almost all of the contents of the upper end of the embryo sac. The pollen tube is seen entering at one side after turning from the micropyle along the outer wall of the nucellus. It contains two red bodies, the "X-Körper" of Nawaschin, in a disorganized condition. The tube is badly ruptured just below the tip. To the right of the pollen tube is the egg nucleus. One male nucleus is just out of the pollen tube and is partly under the egg nucleus. It is curved and is of about the same thickness throughout. At the lower right side of the figure, the upper polar nucleus is seen. Between the egg nucleus and the polar nucleus is a synergid. The long slightly curved male nucleus is seen in the cytoplasm of the "Endospermanlage." It is smaller at the ends but does not have such an appearance of motility as the male nucleus shown in FIG. 2.

FIG. 5 shows a slightly curved male nucleus in the cytoplasm of the "Endospermanlage" almost in contact with the upper polar nucleus. It is slightly curved and tapers back somewhat from the end nearer the polar nucleus. Both ends are rounded. A male nucleus at practically the same stage is shown in FIG. 7. FIG. 6 represents a rather unusual case of this stage. The male nucleus is twisted, and much coiled, and is small at both ends. The end to the left is nearer the polar nucleus and curves beneath the thick central portion.

The contents of all of the male nuclei which are free in the cytoplasm of the cells of the embryo sac show great similarity. A typical, very distinct example is shown in FIG. 7. The chromatin contents are in an irregular network. Usually somewhat larger chromatin granules are present in the network in the central portion of the male nucleus (FIGS. 1, 6, 7). There are apparently no nucleoli to be seen at this stage. The male nuclei stain darker than other nuclei of the sac with the stains used.

After the male nuclei are discharged, there are usually two dark staining bodies to be seen in the end of the pollen tube. These bodies, the "X-Körper" of Nawaschin, often show structure and appear to be more or less disorganized nuclei, but in some instances one or both are entirely lacking in structure. They are of various shapes. Usually, however, one is nearly spherical while the other is larger, ovoid, and often pointed at one end (FIG. 9). In this figure the bodies also show definite nuclear structure. They persist throughout all stages of fertilization and even in young embryo development. That these bodies are nuclei can hardly be questioned. They are probably the two vegetative nuclei corresponding to the vegetative nuclei, (*c*) and (*d*), in FIGS. 3B and 3C.

The male nucleus, when in contact with the egg nucleus, is usually coiled or curved. In some few cases, however, the male nucleus is straightened out when it first comes in contact with the egg nucleus. FIG. 4 shows a long tapering male nucleus with the large end touching the egg nucleus. FIG. 8 shows a male nucleus with the large end in contact with the egg nucleus. Many cases of these examples have been observed and usually the male nucleus is larger at the point of contact with the egg.

Whether the male nucleus reaches the egg nucleus while straightened out or coiled, sooner or later it becomes more or less curved around one side of the egg nucleus. FIGS. 10, 11, 12 and 13 show the male nucleus partly curved around the egg nucleus. In FIG. 13, the side of the egg nucleus is somewhat indented where the male nucleus is in contact. This is not uncommon.

The male nuclei finally lose their long curved shape. They contract somewhat, becoming shorter and more rounded in contact with the egg. In some cases the male nucleus seems to occupy an indentation in the egg (FIG. 15), while in other cases the two sexual nuclei are flattened at the point of contact (FIGS. 16, 17). The male nucleus, on losing its vermiform shape, also loses the fine irregular network of its chromatin contents, characteristic of earlier stages. FIG. 14 shows this condition. The male nucleus has contracted somewhat and its contents consist of large and small granules with some of its earlier net-like structure. The egg nucleus is slightly indented. The chromatin granules of the male nucleus become coarser with finer threads running out from them. Many small pieces of chromatin are also to be seen. A little later in some cases nucleoli appear to be present. FIG. 15 shows the male nucleus after it has lost most of its earlier structure, in an indentation in the egg nucleus. FIG. 16 shows a little later stage than FIG. 15, but with the male and female nuclei flattened at the point of contact. In both cases, the male nucleus stains a little darker. The egg nucleus has very large chromatin granules. In FIG. 16, the male nucleus has very small pieces of chromatin along their common boundary. The male and egg nuclei remain in contact a long while before their common boundary finally disappears and their contents mingle. FIG. 17 shows the male and egg nucleus in contact, each containing a large mass of chromatin and small nucleoli. In FIG. 18 the fusion is almost complete. Most of the contents of the male nucleus are in a thread-like structure. Some of the contents of the egg nucleus are thread-like, but a large part are massed in the center of the nucleus. A large nucleolus is present. The thread-like structure appears to run from one nucleus into the other. In both FIGS. 17 and 18, the male nucleus is smaller than the egg nucleus. FIG. 19 shows a stage where the common boundary has disappeared, the contents have apparently mingled, and those from the male and female nuclei are not to be distinguished, but there is still an indication of the outlines of the two nuclei. A similar stage is shown in FIG. 20. In this case the chromatin contents of the fused nuclei are more irregular.

In most cases it is not until the male nucleus and the egg nucleus have completely fused that we find any appearance of the formation of the spireme. In rare cases, however, the spireme stage is found while the two nuclei are still distinct in outline. A very clear example of this condition is shown in FIG. 21. The spireme thread is rather thick in both nuclei and small nucleoli are still present. It is possible that the nuclear walls have broken down at the point of contact but the nuclei appear to be quite distinct.

Soon after the complete fusion of the egg and the male nucleus, the resulting nucleus becomes more nearly spherical in shape. A spireme is formed. FIG. 22 shows the pear-shaped egg cell with the early spireme of the fertilized egg. The chromatin of the male and female nuclei cannot be distinguished. Delicate threads can be seen at places along the spireme. The thread shortens and thickens until it appears as shown in FIG. 23. Here the spireme is in a late stage—just before the chromosomes are formed. The division of the fertilized egg is normal as is shown in FIGS. 24 and 25. The spindle shows plainly in most cases (FIG. 24), but sometimes it stains lightly (FIG. 25). The figures do not show the entire dividing nuclei but are drawn from sections of the same.

FIG. 25 represents a condition of the egg cell which is characteristic for this and later stages as well as many earlier stages. The egg cell elongates after fertilization. A large vacuole usually forms in the upper part. The fusing sexual nuclei before and after fusion are located in the lower part—lying in a dense cytoplasm. A longitudinal section of the cell shows a more or less crescent-shaped mass of dense cytoplasm, which is well shown in FIGS. 19 and 25.

The two-nucleate embryo is shown in FIG. 26. After division, a definite wall is formed, making two cells, which also appear somewhat crescent-shaped in section. The nuclei are flattened, the short diameter corresponding to that of the cells.

The fusion of the second male nucleus and the two polar nuclei has also been studied. The male nucleus first comes in contact with the upper polar nucleus and together these nuclei migrate to the lower polar nucleus. When the male nucleus comes in contact with the upper polar nucleus, it is usually more or less straightened out. FIG. 27 shows the male nucleus curved along one side of the upper polar nucleus. Several cases were observed where the male nucleus had both ends in contact with the upper polar nucleus (FIG. 29). Sometimes the male nucleus appears to have come in contact with the upper polar nucleus while still coiled. The male nucleus soon shortens and thickens along one side of the polar nucleus (FIG. 28). The contents of the male nucleus are denser and in larger pieces. The polar nucleus is not indented as is often the case of the egg, but often becomes flattened at the point of contact.

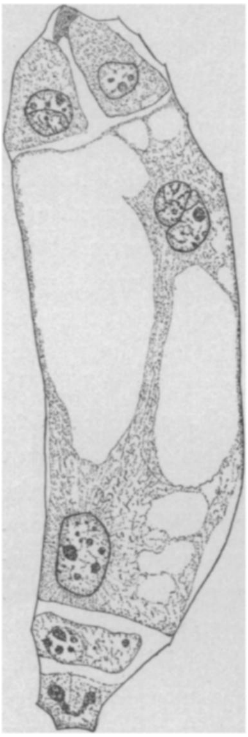

FIG. 2. Embryo sac showing: one male nucleus in contact with the egg nucleus; the other male nucleus and upper polar nucleus in contact and migrating toward the lower polar nucleus.

The contraction and loss of the vermiform shape of the male nucleus usually occurs before the two nuclei migrate to the lower polar nucleus. The male nucleus may, however, retain its vermiform shape during the time of its migration with the upper polar nucleus, or even after the two nuclei have reached the lower polar nucleus. TEXT FIG. 2 shows a longitudinal section of the entire embryo sac with the egg and one of the male nuclei in contact, a synergid, the upper polar nucleus and the second male nucleus in contact, beginning their migration towards the lower polar nucleus, and the antipodal nuclei. The male nucleus in contact with the upper polar nucleus has lost its vermiform shape. The cytoplasm of the upper part of the "Endospermanlage" is massed mostly along one side of the sac and especially around the two migrating nuclei. The strands of cytoplasm and the massing of the contents largely along the one side of the "Endospermanlage" at the upper end is very characteristic.

FIG. 31 shows the migrating nuclei almost in contact with the lower polar nucleus. The male nucleus is on the side nearest the lower polar nucleus and has retained its net-like structure.

Both polar nuclei appear to be in a resting condition. The male nucleus may be attached on any side of the upper polar nucleus.

The cases of migration of the upper polar nucleus together with the male nucleus were not found frequently, only forty-seven cases being found in the great number of fertilization stages. However, the cases of triple fusion of the polar nuclei and male nucleus were very abundant.

TEXT FIG. 3 shows the embryo sac in longitudinal section with a synergid, the egg and male nucleus in contact, and the two polar nuclei and second male nucleus in contact. The male nucleus stains darker and is between the polar nuclei. It will be noted that there is very little cytoplasm in the upper end of the "Endospermanlage" at this time, which is very characteristic. It might appear, on comparing TEXT FIGS. 2 and 3, that the lower polar nucleus migrates also to meet the two approaching nuclei, but if there is a migration of the lower polar nucleus, it is slight, as has been observed in numerous examples, and it is accidental that the two sacs drawn should differ so markedly in this respect.

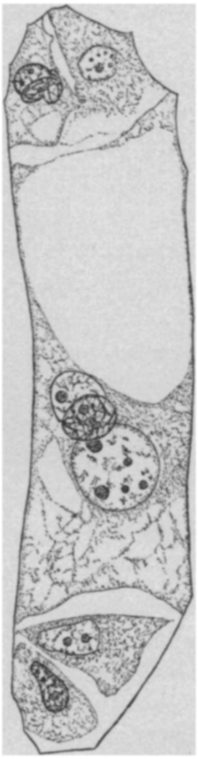

FIG. 3. Embryo sac showing: the remaining synergid; one of the male nuclei coiled and in contact with the egg nucleus; the other male nucleus and upper polar nucleus just reaching the lower polar nucleus.

FIGS. 33 and 34 show triple fusion. In FIG. 34, the male nucleus is at the side, while in FIG. 33, it is between the two polar nuclei. At this stage the chromatin of the male nucleus has lost some of its net-like structure and contains large pieces of chromatin with delicate threads, and in some cases, nucleoli. FIG. 32 shows a case where the two polar nuclei and male nucleus are in contact, but the latter is still vermiform and its contents are in a net-like structure.

The polar nuclei remain in contact without fusing for considerable time. At the time of fusion the chromatin contents are partly thread-like with large irregular pieces of chromatin scattered throughout. FIGS. 35, 36 and 37 show the three nuclei completely fused. In FIG. 37, the outlines of the three nuclei can be seen but their common membranes have disappeared. A fine network is to be noted in the central part of the fused nuclei.

Many cases of complete fusion were observed. There is no question but that the two polar nuclei and the male nucleus completely fuse and that no chromatin is thrown out in the process.

The duration of the spireme must be very short, as only a few very clear spireme stages of the triple fusion, or endosperm nucleus, were found. A clear example of a late spireme stage is shown in FIG. 38. There is an irregular nucleolar mass to the left. At this stage the thread is thickened and apparently approximately equally distributed throughout the nuclear cavity. The thread is just beginning to form chromosomes. The nuclear membrane has disappeared and delicate strands of cytoplasm are in contact with the forming chromosomes.

The cases of first division of the endosperm nucleus were abundant and in every case appeared to be normal, apparently an equal number of chromosomes going to each pole. FIG. 39 shows a very clear anaphase of the first division of the endosperm nucleus. The spindle is very clear in this and most other cases. The spindle is pointed at both ends. The first division of the endosperm nucleus results in the formation of two free nuclei. These divide rapidly and often the four or six nucleate endosperm is formed before the egg and male nuclei are completely fused.

Aberrant fertilizations are occasionally encountered. In three cases both the egg and the remaining synergid were being fertilized. The male nuclei were still distinct. In two of these cases the upper polar nucleus was in its usual position near the egg apparatus, but unfertilized. In the third case the upper polar nucleus had almost reached the lower polar nucleus, having migrated alone.

Two cases were observed where two pollen tubes had entered the ovule, one coming down the micropyle, the other entering the ovule at about the level of the egg apparatus. Only the pollen tube coming down the micropyle had entered the embryo sac.

Besides the fertilization of the egg and polar nucleus there are other processes of interest. Many cases were observed where the contents of the nucleus of the remaining synergid were in the spireme stage. Other stages in the division of the synergid as well as the two-celled stage were observed. No stage of development beyond the two-celled stage was observed.

Several stages were found where the normal antipodal nucleus was dividing. It will be interesting to follow the development of the dividing synergid and the antipodal nucleus through later stages of embryo and endosperm development.

It was noted that the embryo sac becomes longer and curved as it develops. In some stages of early embryo development, it is bent at almost right angles.

DISCUSSION

The cases of the entrance of the pollen tube into the embryo sac, although few, throw some light on problems relating to this process. There is no evidence in *Fritillaria pudica* to show that the synergid empties its contents into the micropyle in order to aid in the entrance of the pollen tube, as has been suggested by several writers. The fact that all the cells of the egg apparatus are intact at the time the tip of the pollen tube is in contact with the wall of the embryo sac excludes such possibilities in *Fritillaria pudica*.

The pollen tube turns along the nucellus and apparently is attracted as close as possible to the nucleus of a synergid before entering. One synergid is practically always destroyed by the entering pollen tube. The suggestion lies close at hand that the synergid is a factor in attracting the pollen tube. The suggestion that the synergid, or parts of it, are used as food by the pollen tube, seems quite tenable.

It is practically certain that in *Fritillaria pudica* the "X-Körper" of Nawaschin are more or less disorganized nuclei. They may be two tube nuclei, but whether there are two tube nuclei formed here has not been determined. Or one of the red bodies may be the disintegrating nucleus of the synergid through which the pollen tube has passed. At all events it is clear that the "X-Körper" of Nawaschin are more or less disorganized nuclei, and not disintegrating masses of cytoplasm from which the male nuclei have escaped, as Welsford (14) has maintained is the case in *Lilium.*

The male nuclei while free in the cytoplasm of the cells of the embryo sac usually do not have the appearance of motility (FIGS. 1, 5, 7). However, the vermiform appearance of some of the male nuclei while free in the cytoplasm and in contact with the female nuclei, is certainly suggestive of motility (FIGS. 1, 2, 6, 11, 13, 27, 30). It has been noted that the male nuclei, while in the pollen tube, are more or less coiled. Yet when seen part way to the nuclei with which they fuse, especially when the distances are long, the male nuclei are quite straight. The uncoiling is also slightly suggestive of motility. The male nuclei in contact with the egg and polar nuclei are seen to curve around the latter, which suggests independent motility and movement on the part of the male nuclei. The large number of stages found at this time as well as the appearance of the male nuclei, suggests that their movement may be rather slow, perhaps a creeping amoeboid motion. It might be suggested that it is possible that the male nuclei may be conveyed to the nuclei with which they fuse, both by their own motility and by the streaming of the cytoplasm.

It is quite certain that the upper polar nucleus and male nucleus in contact, migrate to the lower polar nucleus by cytoplasmic streaming. In most cases the male nucleus has lost its vermiform appearance before migration. Moreover, it is found in contact at any side of the upper polar nucleus. The accumulation of cytoplasm around the migrating nuclei and the small amount left in the upper portion of the "Endospermanlage" at the time of triple fusion, is evidence that the two nuclei are very probably carried by cytoplasmic streaming (TEXT FIGS. 2, 3).

It has been suggested that the rapidity of movement of the male nuclei is evidence of their motility. Yet the migration of the upper polar nucleus with the male nucleus, which appears to be brought about by streaming, even though the latter have further to go, is observed quite as rarely as free male nuclei.

The abundance of material showing the various stages leading to the complete fusion of the male and female nuclei, as well as the numerous examples of complete fusion and subsequent stages in the division of the fertilized egg, leaves no doubt that at least in *Fritillaria pudica* the complete union of the male and female nuclei actually occurs. The rare appearance of such cases as that of the spireme stage in the egg and male nuclei when their outlines are still distinct, is probably of little significance in this respect. It is probable that these nuclei subsequently fuse completely because no later stages of incomplete fusion were found. If the adjacent walls of these two nuclei are not broken down, it would suggest that the fusion of the two sexual nuclei is not necessary to stimulate the formation of the early stages in division. This suggestion is supported by cases found in the Gymnosperms by Miss Ferguson and others.

From the many stages and abundant cases of triple fusion, there is no doubt that the two polar nuclei and the male nucleus fuse completely and that the subsequent division is normal. In this fusion there are at least twice as many female as male chromosomes. If this phenomenon of a normal triple fusion holds true for plants whose hybrids show a Mendelian ratio in endosperm characters, then an interesting question arises as to the distribution of the characters among the chromosomes.

SUMMARY

The results obtained from the study of fertilization in *Fritillaria pudica* may be summarized as follows:

1. In the mature embryo sac of *Fritillaria pudica* the egg cell cannot be distinguished from the other two cells of the egg apparatus. The "Endospermanlage" extends up between and around the cells of the egg apparatus.

2. The pollen tube usually turns along the outer wall of the nucellus or embryo sac before entering. At this time the cells of the egg apparatus are all intact.

3. The pollen tube practically always enters and at least partly destroys one synergid.

4. Although a distinct appearance of motility is evident in many male nuclei, the majority do not suggest much motility.

5. The male nucleus and the egg nucleus fuse completely before division.

6. The upper polar nucleus, with the male nucleus in contact

with it, migrates to the lower one, probably by cytoplasmic streaming.

7. The two polar nuclei and male nucleus fuse completely and the subsequent division is normal.

8. The two red bodies, the "X-Körper" of Nawaschin, which remain in the pollen tube after the male nuclei have been discharged, are very probably nuclei. They probably correspond to the two vegetative nuclei described in the pollen tube before the two male nuclei were discharged.

In conclusion I wish to acknowledge my indebtedness to Dr. C. E. Allen for literature, to Dr. F. L. Pickett for extending the privileges of his laboratory, and especially am I indebted to my wife, Dr. Hally Jolivette Sax, for her constant encouragement and assistance throughout the work.

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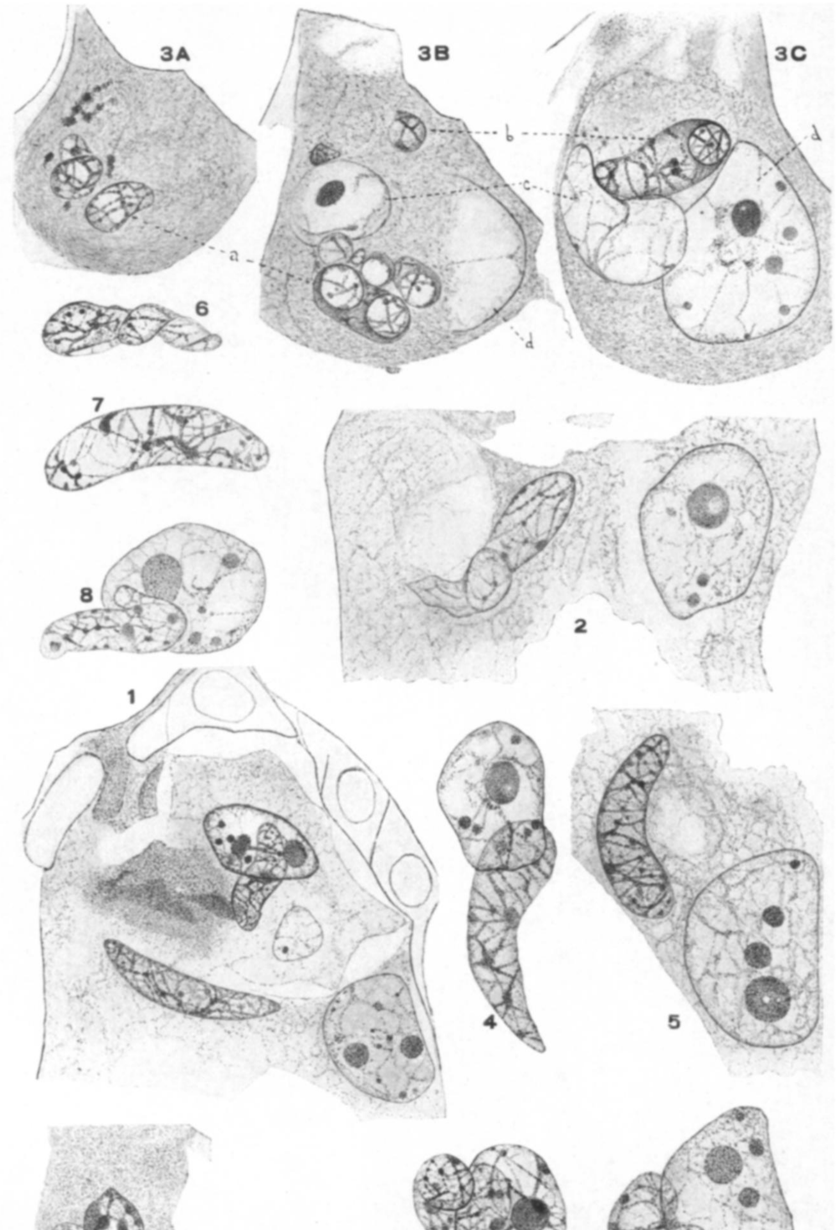

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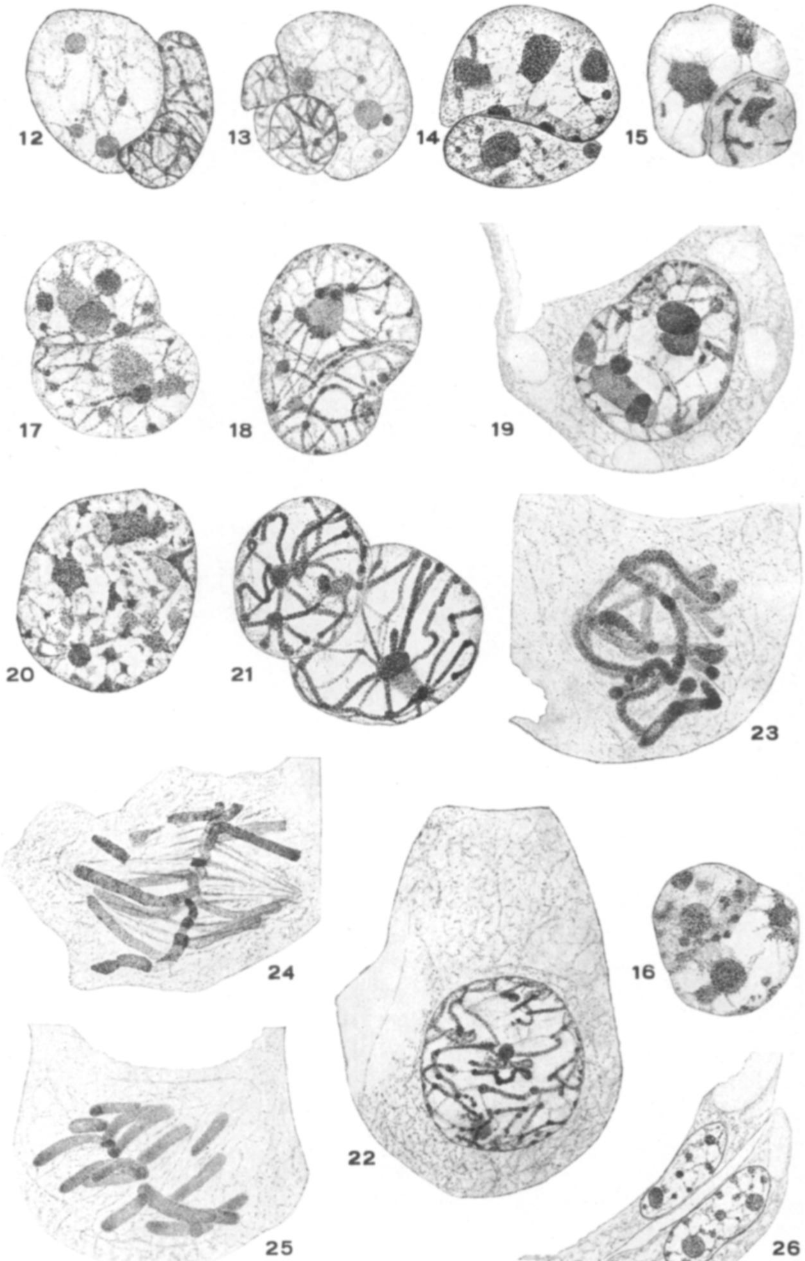

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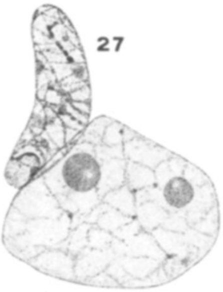
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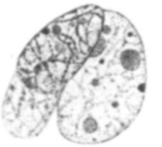
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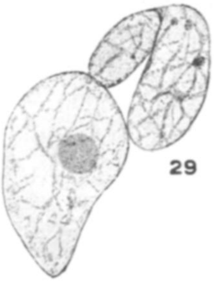
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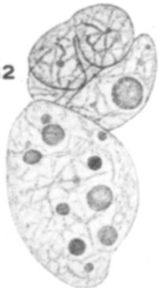
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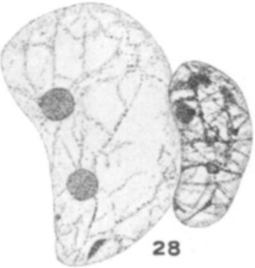
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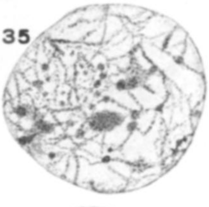
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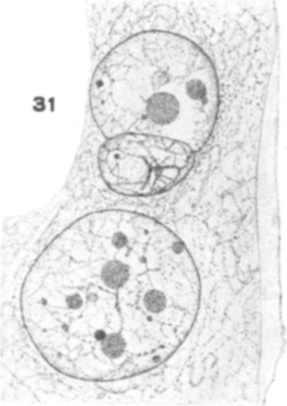
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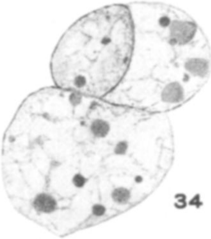
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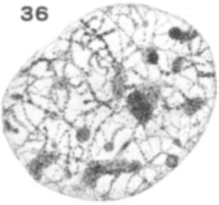
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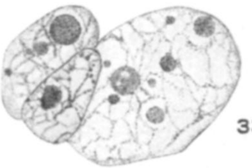
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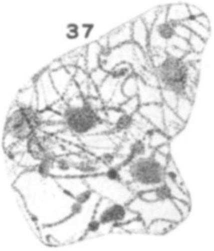
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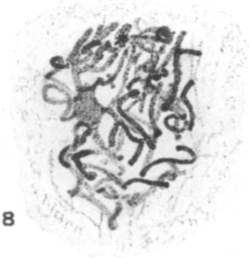
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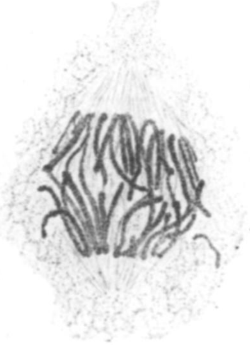
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Explanation of plates 27–29

All drawings were made with the aid of the camera lucida. All figures were drawn from single sections. The magnification is as follows: FIG. 8, 520 diameters; FIGS. 1, 30–39, 720 diameters; FIG. 2, 750 diameters; FIGS. 3–7, 9, 10–29, 1,120 diameters.

PLATE 27

FIG. 1. Micropylar end of the embryo sac in section, showing entrance of pollen tube, with disorganized red bodies—a portion of one synergid—one male nucleus coming in contact with the egg nucleus on the under side, and the second male nucleus in the cytoplasm of the "Endospermanlage" on its way to the upper polar nucleus.

FIG. 2. Section of upper part of embryo sac showing one of the male nuclei on way to polar nucleus.

FIGS. 3*A*, 3*B*, 3*C*. Consecutive longitudinal sections of a pollen tube after entering the embryo sac. The two male nuclei, (*a*) and (*b*), stain darker than the vegetative nuclei. Two vegetative nuclei are shown, (*c*) and (*d*).

FIG. 4. Male nucleus coming in contact with the egg nucleus.

FIG. 5. Male nucleus almost to upper polar nucleus.

FIG. 6. Male nucleus coiled and twisted, on way to upper polar nucleus.

FIG. 7. Male nucleus on way to upper polar nucleus.

FIG. 8. Male nucleus touching the egg nucleus.

FIG. 9. Pollen tube containing the two dark bodies which show nuclear structure.

FIG. 10. Male nucleus coiled and in contact with the egg nucleus.

FIG. 11. Male nucleus in contact with egg nucleus.

PLATE 28

FIGS. 12, 13. Male nucleus in contact with egg nucleus.

FIG. 14. A later stage. The male nucleus has lost its vermiform shape and its earlier net-like structure.

FIG. 15. Male nucleus in indentation of egg nucleus.

FIGS. 16, 17, 18. Male nucleus and egg nucleus fusing.

FIG. 19. Egg cell with male nucleus and egg nucleus completely fused.

FIG. 20. Complete fusion of egg and male nucleus.

FIG. 21. Case of formation of spireme before complete fusion.

FIG. 22. Egg cell containing the fertilized egg in early spireme.

FIG. 23. Egg cell containing the fertilized egg in late spireme.

FIG. 24. Equatorial plate stage in first division of fertilized egg.

FIG. 25. Typical crescent-shaped section of the equatorial plate stage of dividing fertilized egg cell.

FIG. 26. Two-nucleate stage of embryo.

PLATE 29

FIG. 27. Male nucleus in contact with upper polar nucleus.

FIG. 28. A slightly later stage than Fig. 27.

FIG. 29. Male nucleus with both ends in contact with upper polar nucleus.

FIG. 30. Male nucleus in contact with upper polar nucleus while on way to lower polar nucleus.

FIG. 31. Male nucleus and upper polar nucleus almost touching the lower polar nucleus.

FIG. 32. Two polar nuclei and coiled male nucleus in contact.

FIG. 33. Triple fusion. Male nucleus between the polar nuclei.

FIG. 34. Later stage of triple fusion with male nucleus on the side.

FIGS. 35, 36, 37. Endosperm nucleus resulting from the complete fusion of male nucleus and polar nuclei.

FIG. 38. Late spireme stage of endosperm nucleus.

FIG. 39. Early anaphase of first division of endosperm nucleus.